## WEST

## **End of Result Set**

Glu-Arg-Lys, Glu-Pro-Lys, Glu-Lys-Lys,

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## Generate Collection

12: Entry 1 of 1 File: USPT Feb 16, 1999 US-PAT-NO: 5971098 DOCUMENT-IDENTIFIER: US SETUCAS A TITLE: Nematode-extracted anticoagulant protein DATE-ISSUED: February 16, 1999 INVENTOR-INFORMATION: CITY STATE DIP DODE DOUNTRY NAME Vlasuk; George Phillip Carlsbad C.E.  $\Pi \cdot A$ X. A N/AStanssens; Patrick Eric Hugo St-Martens-latem N/ABEX Messens; Joris Hilda Lieven N/AN/ABEX Antwerp N/A N,'ALauwereys; Marc Jozef Haaltert BEX Laroche; Yves Rene Brussels N/AN/ABEX N/A N/ABEK Jespers: Laurent Stephane Tervuren Bansemans; Yanmick Georges Jozef Bredene N/AN,ABEK Moyle; Matthew Escondido -CA N, A N/ACA Bergim; Peter W. San Diego  $N \cdot A$ N/AUS-CL-CURRENT: 514/12; 530/324, 530/350 CLAIMS: We claim: 1. An isolated protein having anticoagulant activity and having one or more Mematode-extracted Anticoaquiant Protein domains ("NAP domains"), wherein each NAP domain includes the sequence: Tys-A1-Cys-A2-Cys-A3-Cys-A4-Tys-A5-Cys-A6-Tys-A7-Tys-A8-Cys-A9-Cys-A10, wherein (a) A1 is an amin: acid sequence of 7 to 3 amino acid residues; (b) A2 is an amin; acid sequence; (c) A3 is an amine acid sequence of 3 amino acid residues; (d) A4 is an amino acid sequence; (e) A5 is an amino acid sequence of 3 to 4 amino acid residues; (f) A6 is an amine acid sequence; (q) A7 is an amino acid residue; (h) As is an amine acid sequence of 11 to 12 amino acid residues; (i) A9 is an amino acid sequence of 5 to 7 amino acid residues; and (f) Ald is an amino acid sequence; wherein each of A2, A4, A6 and A10 has an independently selected number of independently selected amino acid residues and each sequence is selected such that each NAP domain has in total less than about 120 amino acid residues and wherein said isplated protein is derived from a hematophagous nematode species. 1. The protein of claim 1, wherein A3 has the sequence Glu-A3.sub.a -A3.sub.b, wherein A3.sub.a and A3.sub.b are independently selected amino acid residues. :. The protein of claim 1, wherein A3 has the sequence Glu-A3.sub.a -A3.sub.b, wherein A3.sub.a is selected from the group consisting of Ala, Arg, Pro, Lys, Ile, His, Leu, and Thr, and Ad.sub.b is selected from the group consisting of Lys, Thr, and Arg. 4. The protein or claim 4, wherein Arms selected from the drop consisting to Glu-Ala-Lys,

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Glu-Ile-Thr,
Glu-His-Arg,
Glu-Leu-Lys, and
Glu-Thr-Lys.
5. The protein of claim 1, wherein A4 is an amino acid sequence having a net animic
charge.
6. The protein of claim 1, wherein AT is Val.
 . The protein of claim 1, wherein AT is Ile.
8. The protein of claim 1, wherein Af includes the amino abili sequence Af.sub.a
-A8.sub.b -A8.sub.c -A8.sub.d -A8.sub.e -A8.sub.f -A8.sub.g - [$E2. ID. NO. e-],
wherein
(a) A8.sub.a is the first aming adid residue in As,
 'b' at least one of AB., ub.a and AB. sub.b is selected from the group consisting of
Glu or Asf, and
(a) A8.sup.o through As.sub.g are independently selected amini acid residues.
9. The protein of claim 8, wherein
(a) A3.sur.a is Glu or Asp,
(b) AB.sup.b is an independently selected amino acid residue,
(c) AB.sub.c is Gly,
(d) AB.sub.d is selected from the group consisting of Phe, Tyr, and Leu,
(e) A3.sub.e is Tyr,
(f: A3.sur.f is Arg, and
(g \in AB.sub.g is selected from Asp and Asn.
1). The protein of claim 9, wherein -A8.sub.c -A8.sub.d -A8.sub.e -A8.sub.f -A8.sub.g
- is selected from the group consisting of
31y-Phe-Tyr-Arg-Asp [SEQ. IO. NO. 69],
Gly-Phe-Tyr-Arg-Asr [SEQ. IO. NO. 70],
Gly-Tyr-Tyr-Arg-Asp [SED. ID. ND. 71], Gly-Tyr-Tyr-Arg-Asr. [SED. ID. ND. 72], and
Gly-Leu-Tyr-Arg-Asp [SED. ID. NO. 73]
11. The protein of claim 8, wherein
(a) A8.sub.a is an independently selected amino acid residue,
(b Af.sur.b is Rlu or Asp,
    Af.suk.b is Gly,
(d) Af.sub.d is selected from the group consisting of Phe, Tyr, and Leu,
(e) Af.sub.e is Tyr,
(f: A8.sub.f is Arg, and
(g) A8.sub.g is selected from Asp and Asn.
12. The protein of claim 11, wherein -A8.sub.c -A8.sub.d -A8.sub.e -A8.sub.f
-A^{\omega}.sub.g - is selected from the group consisting of
Gly-Phe-Tyr-Arg-Asp [SED. ID. NO. 69],
Gly-Phe-Tyr-Arg-Asn [SED. ID. No. 70],
Gly-Tyr-Tyr-Arg-Asp [SEQ. II. NO. 71],
3ly-Tyr-Tyr-Arg-Asn [SEQ. II. NO. 72], and
Gly-Leu-Tyr-Arg-Asp [SEQ. ID. NO. 73].
13. The protein of claim 8, wherein -A8.sub.c -A8.sub.d -A8.sub.e -A8.sub.f -A8.sub.g
- is selected from the group consisting of
Gly-Phe-Tyr-Arg-Asp [SEQ. ID. No. 63],
Gly-Phe-Tyr-Arg-Asn [SEQ. ID. NO.
Gly-Tyr-Tyr-Arg-Asp [SEQ. ID. NO. 71],
Sly-Tyr-Tyr-Arg-Asn [SEQ. ID. NO. 72], and
Bly-Leu-Tyr-Arg-Asp [SEQ. II. NO.
14. The protein of claim 1, wherein A10 includes an amino acid sequence selected from
the group consisting of
Glu-Ile-His-Val (SEQ. ID. NO. 74), Asp-Ile-Hie-Met-Val (SEQ. ID. NO. 78),
Phe-Ile-Thr-Phe-Ala-Pro [SEQ. II. NO. 76], and
Met-Glu-Ile-Ile-Thr [SEQ. II. NO. 77].
15. The protein of claim 14, wherein AlO includes the amino acid sequence
Glu-Ile-Ile-His-Val [SEQ. ID. NC. 74].
16. The protein of claim 15 having a NAP domain with an amino acid sequence of a NAP
domain of AcaNAP6 (SEQ. ID. NO. 41).
17. The protein of claim 14, wherein AlS includes the amino acid sequence
Asp-Ile-Ile-Met-Val [SEQ. ID. NO. 75].
18. The protein of claim 17 having a NAF domain with an amino acid sequence of a NAF
domain of AdaMAP48 (SEQ. ID. NO. 42).
19. The protein of slaim 14, wherein AlC includes the sequence the Thr-Fhe-Ala-Fre [SEQ. II. MC. 76].
20. The protein of claim 19 having a NAF domain selected from a NAF domain of
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AdaMARIS SEQ. II. No. 45 , a MAR dimain if AraMAR.4 CE. II. NO. 44 , a MAR dimain of AdaMARIS SEQ. II. No. 46 , a MAR dimain of AraMARAS SEC. II. No. 47 , a MAR dimain of AraMARAS SEC. II. No. 47 , and a MAR domain of AraMARAS SEC. II. No. 47 , and a MAR domain of AraMARAS SEC. II. No. 47 , and a MAR domain of AraMARAS SEC. III. No. 47 , and a MAR domain of AraMARAS SEC. III. No. 47 , and a MAR domain of AraMARAS SEC. III. No. 47 , and a MARAS SEC. III. No. 48 , and a MARAS SEC. III. NO. 48
  or 49 .
  21. The protein of blaim 14, wherein All includes the sequence Met-314-114-114-Th:
   [SEQ. ID. NO. 77]
  22. The protein of claim 21 having a MAP domain with an amino acid sequence selected
 from a sequence of a NAP domain of AcaNAP45 (SEQ. ID. NOS. 81 or 83%, a NAP domain of AcaNAP47 (SEQ. ID. NOS. 81 or 84%, a NAP domain of AduNAP7 (SEQ. ID. NOS. 82 or 84%, a NAP domain of AcaNAP8 (SEQ. ID. NOS. 82), id. NOS. 88 , a NAP domain of AcaNAP8 (SEQ. ID. NO. 85),
  and a NAP dimain of AbeNAPT (SEQ. ID. NO. 55%.
  23. The protein of claim 1, wherein said nematode species is selected from the group
   ponsisting of Ancylostoma caninum, Ancylostoma ceylanicum, Ancylostoma ducdenalé,
  Necator americanus, and Heligomosomoides polygyrus.
  24. The protein of claim 1, wherein
   (a) A3 has the sequence Glu-A3.sub.a -A3.sub.b, wherein A3.sub.a and A3.sub.b are
  independently selected amino acid residues;
   (b) A4 is an amino acid sequence having a net anioric charge;
         A7 is selected from the group consisting of Val and Ile;
    d A8 includes an amino acid sequence selected from the group consisting of
 Sly-Phe-Tyr-Arg-Asp [SEQ. ID. NO. 69], Sly-Phe-Tyr-Arg-Asn [SEQ. ID. NO. 70], Sly-Tyr-Tyr-Arg-Asp [SEQ. ID. NO. 71], Sly-Tyr-Tyr-Arg-Asn [SEQ. ID. NO. 72], and Sly-Lea-Tyr-Arg-Asp [SEQ. ID. NO. 73]; and
   (e) All includes an amino sequence selected from the group consisting of
 Glu-Ile-Ile-His-Val [SEQ. II. No. 74],
 Asp-Ile-Ile-Met-Val [SEQ. II. NO. 75],
 Phe-Ile-Thr-Phe-Ala-Pro [SEQ. ID. NO. 76], and
 Met-Glu-Ile-Ile-Thr [SEQ. ID. ND. 77].
 25. The protein of claim 24 having a NAP domain selected from the group consisting of
 a NAP domain of AcaNAP6 (SEQ. ID. NO. 41), a NAP domain of AcaNAP48 (SEQ. ID. NO.
 41), a NAP domain of AbaNAP23 \pm 3EQ. ID. NO. 43), a NAP domain of AbaNAP24 (SEQ. ID.
 MO. 44), a NAP domain of AcaNAP25 (SEQ. ID. NO. 45), a NAP domain of AcaNAP44 (SEQ.
  10. NO. 45:, a NAP domain of AdaNAP31 (SEQ. ID. NO. 47), a NAP domain of AdeNAP4
 (SEQ. ID. MOS. 43 or 43), a NAP demain of AdaNAP45 (SEQ. ID. NOS. 50 or 53), a NAP
 domain of AbaNAP47 (SEQ. ID. NOS. 51 or 54), a NAP domain of AduNAP7 (SEQ. ID. NOS.
 EL or 56), a NAP domain of AduNAP4 (SEQ. ID. NO. 55 , a NAP domain of AceNAP5 (SEQ.
 IF. NO. 57^{\circ}, and a NAP domain of AceNAP7 (SEQ. ID. NO. 58).
 26. The protein of claim 25, wherein said hematode species is selected from the group
 consisting of Ancylostoma caninum, Ancylostoma deylanicum, Ancylostoma duodenale,
 Medater americanus, and Heligomosomoides polygyrus.
 d7. The protein of claim 1, wherein
  (a) At is selected from the group consisting of
 Glu-Ala-L∵a,
Glu-Ara-Lys,
 Glu-Pro-Lys,
 Glu-Lys-Lys,
Glu-Ile-Thm,
Glu-His-Arg,
Glu-Leu-Lys, and
Glu-Thr-Lys;
  b) A4 is an amino acid sequence having a net anionic charge;
  c) A7 is Val or Ile;
  d) AE includes an amino acid sequence selected from the group consisting of
A8.sub.a -A8.sub.b -3ly-Phe-Tyr-Arg-Asp (SEQ. ID. NO. 78],
A8.sub.a -A8.sub.b -Gly-Pre-Tyr-Arg-Asn [SEQ. ID. NC. 73],
A8.sub.a -A8.sub.b -Gly-Tyr-Tyr-Arg-Asp [SEQ. ID. NO. 80],
A6.sub.a -A8.sub.t -Gly-Tyr-Tyr-Arg-Asn [SEQ. ID. NO. 81], and
A8.sub.a -A8.sub.b -Gly-L+u-Tyr-Arg-Asp [SEQ. ID. NC. 82], wherein at least one of
A8.sub.a and A9.sub.b is 31u or Asp;
(e) A9 is an amino acid sequence of five amino anii reslices; and (f) A10 includes an amino acid sequence selected from the droup consisting of Glu-Ile-Ile-His-Val (CE, 11. Mo. Ta), Asp-Ile-Ile-Met-Val (CE, 11. Mo. Ta), Phe-Ile-Thr-Phe-Ala-Fro (CEQ, 11. Mo. T(), and Met-C3. Tip-Ile-The-Be-sepace to the constant of th
Met-Glu-Ile-Ile-Thr (SEQ. ID. NO.
28. The protein of claim 27 having a NAF domain selected from the group consisting of
a NAP domain of AbaNAF6 (SEQ. ID. NO. 41), a NAP domain of AbaNAP49 (SEQ. ID. NO.
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40', a NAP domain of AdaNAP23 (SEQ. ID. NO. 43', a NAP domain of AdaNAP14 (SEQ. ID. NO. 44', a NAP domain of AdaNAP44 (SEQ. ID. NO. 48', a NAP domain of AdaNAP44 (SEQ. ID. NO. 46', a NAP domain of AdaNAP41 (SEQ. ID. NO. 46'), a NAP domain of AdaNAP41
 SEQ. ID. MIS. 48 or 48, a MAP domain of AbaMAP48 SEQ. ID. MIG. 8. or 68, a MAP domain of AbaMAP48 SEQ. ID. MIG. 8. or 68, a MAP domain of AdaMAP4 SEQ. ID. MIS. 52 or 56), a MAP domain of AdaMAP4 SEQ. ID. MO. 88), a MAP domain of AdaMAP6 SEQ. ID. MO. 88), a MAP domain of AdaMAP6 SEQ. ID. MO. 88).
 29. The protein of claim 27, wherein said nematode species is selected from the group
 consisting of Ancylostoma caninum, Ancylostoma ceylanicum, Ancylostoma duodenale,
 Medator americanus, and Helig.mostmoides polygyrus.
 3). A pharmaceutical composition comprising a protein of claim 1.
 31. A pharmaceutical composition comprising a probability 51977 4.
3. A pharmaceutical composition comprising a probability follows: 3. A method or inhibiting blood coagulation comprising administering a protein or
 chaim I with a pharmaceutically a meptable carrier.
34. A method of inhibiting blood doagulation comprising administering a protein or
 claim 34 with a pharmaceutically acceptable carrier.
 35. A method of inhibiting blood coagulation comprising administering a protein of
 claim 17 with a pharmaceutically acceptable carrier.
 if. A protein of claim 1, wherein said protein has two NAP domains.
 37. A protein of claim 24, wherein said protein has two NAP domains. 38. A protein of claim 27, wherein said protein has two NAP domains.
 39. An isolated protein havin; anticoagulant activity selected from the group sinsisting of AcaMAP6 (SEQ. II. NI. 41), AcaMAP48 (SEQ. ID. NO. 42), AcaMAP23 (SEQ. ID. NO. 42), AcaMAP48 (SEQ. ID. NO. 43), AcaMAP48 (SEQ. ID. NO. 45), AcaMAP48 (SEQ. ID. NO. 
ID. NO. 43 , AdaNAP24 (SEQ. ID. NI. 44), AdaNAP25 (SEQ. ID. NO. 45), AdaNAP44 (SEQ. ID. NO. 46), AdaNAP31 SEQ. ID. NI. 47), AdaNAP4 (SEQ. ID. NO. 62), AdaNAP45 (SEQ. ID. NO. 63), AdaNAP47 (SEQ. II. NO. 64), AdaNAP7 (SEQ. ID. NO. 65), AdaNAP4 (SEQ. ID.
NO. 55', AdeNAP5 (SEQ, ID. NO. 5'', and AdeNAP7 (SEQ. ID. NO. 58).
41. A pharmaceutical composition comprising a protein having a MAP domain selected
from the group consisting of a MAP domain of AcaNAP6 (SEQ. 10. NO. 41), a NAP domain
from the group consisting of a NAP domain of AdaNAP6 (SEQ. ID. NO. 41), a NAP domain of AdaNAP48 (SEQ. ID. NO. 42), a NAP domain of AdaNAP48 (SEQ. ID. NO. 42), a NAP domain of AdaNAP26 (SEQ. ID. NO. 45), a NAP domain of AdaNAP26 (SEQ. ID. NO. 45), a NAP domain of AdaNAP31 (SEQ. ID. NO. 47), a NAP domain of AdaNAP31 (SEQ. ID. NO. 47), a NAP domain of AdaNAP44 (SEQ. ID. NOS. 48 or 49), a NAP domain of AdaNAP46 (SEQ. ID.
MOS. 50 or 53, a NAP domain of AbaNAP47 (SEQ. ID. NOS. 51 or 54), a NAP domain of
AduNAPT (SEQ. ID. NOS. 52 or 58), a NAP domain of AduNAP4 (SEQ. ID. NO. 55), a NAP domain of AdeNAPT (SEQ. ID. NO. 58).
41. A method of inhibiting blood coagulation comprising administering a protein
having a NAP domain selected from the group consisting of a NAP domain of AcaNAP6
(SEQ. II. NO. 41), a NAP domain of AdaNAP43 (SEQ. IE. NO. 42:, a NAP domain of
AdaNAP23 (SEQ. ID. NO. 43), a MAP domain of AdaNAP24 (SEQ. ID. NO. 44), a NAP domain
of AcaNAP25 (SEQ. ID. NO. 45), a NAP domain of AcaNAP44 (SEQ. ID. NO. 46), a NAP
demain of AsaNAP31 (SEQ. IE. NO. 47), a NAP domain of AseNAP4 (SEQ. ID. NOS. 48 and
49), a NAP domain of AdaNAP45 (SEQ. ID. NOS. 50 and 53), a NAP dimain of AdaNAP47
 SEQ. ID. NOS. 51 and 54), a MAP demain of AduNAP7 (SEQ. ID. NOS. 52 and 561), a NAP
domain of AduNAP4 (SEQ. II. NO. 55), a NAP domain of AdeNAP5 (SEQ. ID. NO. 57), and a
NAP dimain of AdeNAP7 (SEQ. 10. No. 58).
42. A protein having two NAP domains, wherein said protein is selected from the group
consisting of AdeNAP4 [SEQ. ID. NO. 62], AdaNAP45 [SEQ. ID. NO. 63], AdaNAP47 [SEQ.
ID. NO. 64], and AduNAP7 [SEQ. ID. NO. 65].
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